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OFFICE OF  
PESTICIDES AND TOXIC SUBSTANCES

Subject: Akin, Gump, Strauss et al submission dated  
3/11/88. Review and Evaluation of Testing Protocol for  
Chlorine Generators Applied to Fresh Fruit (No MRID  
No., RCB No. 3513).

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Frupac Ltda. has submitted for review a testing protocol for chlorine generators applied to fresh fruits. This submission is a follow-up to the pre-registration meeting attended by representatives of EPA, RD and HED, and registrant representatives from Frupac, Ampro and Akin, Gump (see memo of February 23, 1988, R. Quick).

Deficiencies in the Proposed Protocol

1. The total chlorine in grapes after chlorine gassing should be determined after exposure to 40 ppm concentration for 20 minutes rather than 20 ppm, since 40 ppm would be equivalent to the exposure proposed for commerce.

2. Grapes packed with pads containing calcium hypochlorite should be fumigated with chlorine gas at 40 ppm for 20 minutes prior to packing in order to simulate conditions proposed for commerce.

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3. Under "Sampling Procedures," item #4, samples also should be taken at 0 hours after removal of pad.

4. For comparison of total residues of grapes exposed to a single chlorine fumigation and grapes packed with pads, the grapes packed with pads should be fumigated with chlorine gas prior to packing with the pads. In addition, the samples should be stored at the same temperature and for the same length of time.

5. Samples of bruised and shattered grapes must be analyzed also to determine whether a difference in residue concentration occurs.

6. Analytical method validation protocols should be included.

### Conclusions and Recommendations

The questions and concerns raised under "Deficiencies in the Proposed Protocol" should be addressed prior to initiation of the study.

### Detailed Discussion

The registrants wish to provide EPA with data to support the use of slow release pads containing calcium hypochlorite in grape shipping containers. Chlorine gas would be slowly released from the pads and is intended to maintain grape quality during shipping. The use would be analogous to the current use of sulfur dioxide/sulfite salts on grapes.

Chlorine gas is presently used in water treatment. Sodium hypochlorite is GRAS under 40 CFR 180.2(a). Calcium hypochlorite is exempt from the requirement of a tolerance for post-harvest on potatoes (40 CFR 180.1054). Calcium hypochlorite is cleared as an inert ingredient under 40 CFR 180.1001 (c)(d) as a sanitizer and bleaching agent. There are also a number of FDA clearances for sodium hypochlorite under 21 CFR.

In light of the many clearances for hypochlorites, RCB felt that an exemption from tolerance might be appropriate for this use and deferred to TOX Branch, HED, as to what data would be needed.

Toxicology Branch wants residue data for total organic halide, trihalomethane and inorganic halides on both treated and untreated grapes. No toxicology studies have been requested at this time.

The registrant was instructed, during the pre-registration meeting, to conduct residue studies reflecting a "worst case" situation and that the protocols used in the sulfite grape residue studies could be used as models. A validated analytical method of adequate sensitivity is also needed.

The registrant states that the chlorine generator pads are double compartment paper pouches containing calcium hypochlorite. These pads are designed to release gaseous chlorine inside each box of grapes to prevent decay, mainly from the fungus botrytis cinerea.

The stated objectives of the protocol are:

1. To determine the total chlorine content of grapes packed with a chlorine generator pad and stored under normal handling conditions as a function of time.
2. To determine the variation in the total chlorine content of grapes after pad removal as a function of temperature and time.

The grapes will be harvested from California vineyards. Both Thompson Seedless and Flame Seedless grape varieties will be tested in this study. The harvested grapes will be transported to the packing house in open boxes at ambient air temperature. The open boxes of grapes will be fumigated with chlorine gas at room temperature at a concentration of 40 ppm for 20 minutes. The grapes will be selected and packed as follows:

A polyethylene liner vented with 6 mm diameter holes in a 10 cm square pitch pattern is placed in a wooden box measuring 18 5/8" x 11 1/2" x 5 3/4" (8.2 kg. capacity).

Fine wood shavings are placed on the bottom of the polyethylene liner.

A sheet of tissue is placed over the shavings and over the side of the box.

Bunches of tissue-wrapped grapes are placed in the box and the tissue already in the box is folded over the top of the grapes.

The chlorine pad is placed over the tissue.

The polyethylene liner is folded over the pad, and the box is sealed.

The boxes of grapes then will be stored under refrigeration at 0-2° C for up to 40 days.

The sampling procedures would measure total organic chlorine of untreated grapes at room temperature on the day of harvest; determine total chlorine in grapes after gassing at 20 ppm for 20 minutes, analyses to be conducted at room temperature at 0, 2, 4, 8 and 12 hours after gassing; measure total chlorine content of grapes packed with chlorine pad, stored under refrigeration at 0-2° C and analyzed 5, 10, 15, 20, 30 and 40 days after storage; determine variation and depletion of total chlorine content of grapes after pad removal by analyzing 20 and 40 days after initiation of cold storage, with analyses occurring 2, 4, 8 and 12 hours after pad removal in cold storage and at room temperature. In addition, after 20 and 40 days in cold storage sealed grape boxes will be transferred to room temperature for 24 hours prior to analysis at room temperature 0, 2, 4, 8 and 12 hours after the boxes are opened and the pads are removed.

An air sample will be taken from each box prior to opening. Grape samples will be taken from the top 1/4 of the box, ie grapes closest to the pad. All grape analyses will be performed in triplicate. Samples will be stored in airtight Mason jars, shipped to the laboratory in dry ice where they will be placed into a freezer at -17 to -20° C until they are analyzed.

Since the exposure to chlorine gas under normal fumigation conditions would be at 40 ppm concentration level for 20 minutes, the studies which simulate gas fumigation should be conducted at 40 ppm rather than 20 ppm concentration. Also, if the petitioner intends to draw a comparison of chlorine residue levels present in grapes exposed to a single fumigation and those stored with pads, the latter samples should be fumigated with chlorine gas first prior to storing with pads. Although this may be the intent of the petitioner, it is not clearly stated. Similarly it is assumed that for the short and long term studies the grapes will be fumigated prior to storage with pads. Also, under Sampling Procedures, in order to simulate a "worst case" situation the grapes should be sampled at 0 hours after removal of the pad, as well as 2, 4, 8 and 12 hours. Selection of berries for analysis should not be limited to "perfect" berries. Bruised and shattered berries should be sampled in order to determine whether a concentration of residues occurs.

All samples collected will be analyzed for total chlorine residues, ie total organic chlorine, to be determined as total halogenated organics (TOX) and trihalomethane (THM), and inorganic chloride.

Samples for THM and total halogenated organics will be placed in a pint Mason jar. The net weight of the grapes is recorded. Average sample size is approximately 150-250 grams. The jar is immediately sealed and placed in dry ice for shipment and then stored at the laboratory at approximately -20° C until

processing. The sample jars are fitted with a blender blade assembly, and placed in a 0° C environment to stabilize for 10 minutes. The samples are blended briefly (approximately 2-5 seconds). Immediately after blending, the homogenized sample is weighed into containers for analysis.

EPA Volatile Halocarbon method 5020/8010 is performed directly on approximately 5 grams of sample weighed into a 20 ml headspace sample vial which is immediately sealed after filling and then is kept frozen at approximately -20° C until analysis. These samples are prepared in triplicate to make reanalysis possible. The analysis will follow EPA method 5020 (headspace) 8010 Volatile Halocarbons as described in SW846.

Total halogenated organics (TOX) will be analyzed using the following procedure: A weighed amount of homogenate (approximately 20 grams) is weighed into a 40 ml septum vial of hexane immediately after preparation. Five ml of hexane is added to the vial. The vial is shaken in a mechanical shaker for 10 minutes. The vials are placed in an ultrasonic waterbath and processed at 300 watts for 5 minutes. A centrifuge is used to separate the phases efficiently at the end of the processing. The hexane extract is then removed and analyzed.

Analyses of the extract will be achieved using a GC equipped with a Hall Detector. The detector is set up on the Halogen mode as described in Method 8010. The detector response is proportional to the amount of halogens introduced. The detector set up in this manner is non-reactive to other constituents.

Injection of 1 ml of hexane produces a single integratable peak representing total organic (extractable) halide. This procedure will be validated by spiking the samples with known quantities of chloroform, THM mixtures, heptachlor and chlordane covering the range from very light to (low boiling) to very heavy (high boiling). The method is anticipated to yield reproducible results at the 10 ppb detection limit level.

Inorganic chloride will be tested using a chloride ion selective electrode. The electrode will be placed directly into the macerated grapes. The detection limit level resulting from this procedure is anticipated to be on the ppm level.

The petitioner does not state what recovery studies will be run in order to validate these analytic methods in a grape matrix. A protocol for such validation procedures is needed.

cc: R.F., Circu, Haeberer, TOX, PP#7E3473, FDA, PMSD/ISB, RD  
RDI:R. Quick, 4/14/88; K. Arne, 4/14/88; eth